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# SOME OBSERVATIONS REGARDING "OLEUROPEIN" FROM OLIVE LEAVES

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Quite recently Bourquelot and Vintilesco have brought to notice a product obtained by them from the leaves and fruit of the olive tree, the preparation and characters of which they have described in a communication bearing the following title: *Sur l' "oleuropéine," nouveau principe de nature glucosidique retiré d'olivier (Olea europaea, L.). Compt. rend. de l'Acad. des Sciences*, 1908, **147**, pp. 533-535; *J. Pharm. Chim.*, 1908, **28**, pp. 303-314; also this *Journal*, Nov. 7, 1908, p. 588.

In the beginning of their communication the above-mentioned authors state that they have specially considered a paper published some years ago by G. Körner (*Gazz. chim. ital.*, xviii., 1888, p. 209), in its relation to the investigation of a crystalline substance designated olivil, this substance having first been isolated in 1816 by Pelletier from a gum-resin that exudes spontaneously from the bark of the olive tree. They remark further that, although Körner had shown olivil not to be a glucoside, he suggested that it might be a decomposition product of a glucoside occurring in the plant, but was not successful in isolating such a substance. In this connection it may, however, be noted that olivil, $C_{20}H_{24}O_7$, has more recently been examined by Körner and Vanzetti (*Atti R. Accad. Lincei*, 1903 [v.], **12**, 1, 122), and that the occurrence of the latter compound appears to be restricted to the above-mentioned exudation from the olive tree, for our investigations have indicated that it is not a constituent of either olive bark or olive leaves (*Journ. Chem. Soc.*, 1908, **93**, pp. 891-917).

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The unsuccessful attempt on the part of Körner to isolate a glucoside from the olive tree led Bourquelot and Vintilesco to employ their so-called bio-chemical method, which, they state, would quickly enable them to decide whether there existed in the plant a glucoside hydrolysable by emulsin. A preliminary experiment with olive leaves having afforded them indications of the presence of a substance of such a nature they extended their experiments to the bark and the fruit, the material having been collected at different times of the year. In accordance with the results of these observations they concluded that a glucoside exists in different parts of the plant, but in amounts which varied in the respective organs and with the period of vegetation. Bourquelot and Vintilesco then sought to isolate the substance in question, both from the leaves and the young fruit of the olive tree, and state to have succeeded in separating a considerable amount of a product which, although not obtained in a crystalline state, could, nevertheless, be regarded as relatively pure and of a glucosidic nature. To this product they have assigned the name "oleuropein."

The amount of "oleuropein" obtained by Bouquelot and Vintilesco from fresh olive leaves corresponded to 0·75 per cent. of their weight, whereas the young fruit yielded 2 per cent. The product from the leaves they considered to be somewhat less pure than that from the fruit, since it was somewhat darker in colour, more hygroscopic, and had a slightly lower rotatory power, namely $[\alpha]_D = -120°$, as compared with $-127·9°$. The fact, however, that the products from both the leaves and the fruit possessed pretty nearly the same properties, was regarded as evidence of their relative purity.

It appears desirable that in this place consideration should be given to some comments by Bourquelot and Vintilesco on the observations of the present authors respecting the occurrence of glucosidic substances in olive bark. They remark in this connection as follows :—"With regard to the quite recent investigations of Power and Tutin, however interesting in other respects, they have not advanced the question which has engaged our attention, for the fact of having obtained *d*-phenylglucosazone from the products of the action of dilute sulphuric acid on an extract of the bark cannot be considered as proof of the existence of a glucoside. Moreover, the authors do not speak of this in their conclusions."

The above statement would appear to convey the impression that the present authors had subjected a crude extract of olive bark, which was shown to contain a quantity of sugar, to the action of dilute sulphuric acid, and had then merely identified glucose among the products. A perusal of our papers will naturally suffice to show that such an assumption would be totally incorrect. In our investigation of olive bark, for example, to which Bourquelot and Vintilesco specially refer, it was clearly stated in connection with the examination of a brown resin (*loc. cit.* pp. 913-914) that this "as first obtained, was a heavy, brown, viscid syrup," and that "*after being completely freed from substances soluble in cold water*," it was extracted successively with various solvents. Both the ethyl acetate and the alcohol extracts of this resinous substance were subsequently heated with dilute alcoholic sulphuric acid, and in both cases glucose was identified in the resulting products. These results were expressed in the following statements (*loc. cit.*, p. 914): "It was thus evident that some material of a glucosidic nature was present in the ethyl acetate extract. The remaining portion of the brown resin, *which was soluble only in alcohol*, amounted to about one third of the whole. . . . When treated with dilute alcoholic sulphuric acid, *it yielded a quantity of dextrose*, but the only other product was an amorphous resin." This glucosidic character of certain products from olive bark was also referred to in the summary of the investigation (*loc. cit.*, p. 917) in the following words:—"*The portion of the alcoholic extract which was soluble in hot but not in cold water consisted for the most part of brown, resinous products, some of which* (*i.e.*, the ethyl acetate and alcohol extracts) *yielded glucose on hydrolysis.*"

The observations above noted thus render it evident that, contrary to the assumption of Bourquelot and Vintilesco, we had not only afforded proof of the existence of material of a glucosidic nature in olive bark, but had also demonstrated that at least two such substances were present. Substances of a similar nature were likewise observed in the course of our investigation of olive leaves, although not specially noted in the account of that work. In any case we should not have considered it desirable to assign a distinctive name to amorphous, resin-like products, possessing none of the attributes of homogeneity.

The characters ascribed by Bourquelot and Vintilesco to the "oleuropein" obtained by them from fresh olive fruit are briefly as follows:—A yellowish, hygroscopic powder, quite readily soluble in cold water and in hot alcohol, insoluble in ether, and possessing a strongly bitter taste. $[\alpha]_D = -127{\cdot}9°$ (3·026 grammes dissolved to 150 C.c.). The aqueous solution is coloured yellow by alkalis, blood-red by concentrated sulphuric acid, and green by ferric chloride. It is incompletely precipitated by basic lead acetate. The substance reduces Fehling's solution, and more strongly after heating with dilute sulphuric acid.

Although our previous investigation of the constituents of olive leaves and bark (*loc. cit.*) enabled us to feel convinced that the product designated as "oleuropein" could not be, in the chemical sense, an even approximately pure substance, some experiments have been undertaken which not only confirm this conviction but afford definite proof of its heterogeneous character.

## Experimental.

For the purpose of obtaining a product corresponding to the "oleuropein" of Bourquelot and Vintilesco, a portion of the same alcoholic extract of olive leaves was employed as that which had previously served for our investigation of the constituents of these leaves (*Journ. Chem. Soc.*, 1908, **93**, 891). In the preparation of the product the method described by the above-mentioned authors (*loc. cit.*) was followed as closely as possible in all essential details.

A quantity (2000 grammes) of an alcoholic extract, representing 4360 grammes of air-dried olive leaves, was mixed with a considerable volume of water, 150 grammes of calcium carbonate added, and steam passed into the mixture until all the alcohol had been removed. The mixture was then filtered, and the undissolved residue extracted twice successively with water in a similar manner. The combined aqueous liquids were then evaporated in the presence of calcium carbonate until a thick extract was obtained. The latter was extracted with four successive portions, of 1000 C.c. each, of 95 per cent. alcohol. On allowing the liquids obtained by the first two extractions to stand, some mannitol separated, and, after removing the latter, the combined alcoholic liquids were evaporated. A soft extract was thus obtained, which was extracted many times with ethyl acetate saturated with water. These extracts were united and evaporated, when a residue was obtained which was treated with boiling water, and a small amount of undissolved, green, resinous matter removed by filtration. The filtered, aqueous liquid was concentrated under diminished pressure to the consistency of a soft extract, and this was treated with cold, 95 per cent. alcohol, which, however, effected no separation. The alcohol was therefore completely removed, and the dry residue extracted several times with dry ethyl acetate, the combined liquids being subsequently evaporated to dryness. This residue was finally dissolved in absolute alcohol, the solution evaporated, and the resulting syrup dried, first on the water-bath, and then in a desiccator. A brownish, resin-like product was thus obtained, which amounted to 50 grammes, corresponding to 1·15 per cent. of the weight of air-dried olive leaves. It could be reduced to a light yellow powder, but on exposure to the air absorbed moisture and became sticky.

The product obtained as above described possessed all the general properties of the "oleuropein" of Bourquelot and Vintilesco. Thus it had a strongly bitter taste, reduced Fehling's solution, and its aqueous solution showed the previously mentioned behaviour towards alkalis, concentrated sulphuric acid, and ferric chloride respectively.

For the determination of the optical rotation, approximately 1 Gm. of the product was dissolved in 50 C.c. of hot water, and the solution allowed to cool. As the liquid then became somewhat turbid, it was filtered, after which the rotation in a 2 dcm. tube was found to be $\alpha_D = -3^\circ\ 31'$. On evaporating 10 C.c. of the solution a residue was obtained which, after drying at 115° C., weighed 0·1924 gramme. The specific rotatory power of the product was therefore $[\alpha]_D = -91{\cdot}4^\circ$. This is somewhat lower than that observed by Bourquelot and Vintilesco for the "oleuropein" obtained from fresh olive leaves, namely $[\alpha]_D = -120^\circ$.

In order to ascertain whether the product corresponding to "oleuropein" is homogeneous, the following experiments were conducted. A quantity (8 grammes) of the above-described product was boiled with 25 C.c. of water, and the liquid allowed to cool, after which the upper, aqueous layer was decanted from a lower layer of syrupy, undissolved material, and filtered. This operation was repeated five times successively with the undissolved portion of material, when about 2 grammes of the product still remained undissolved. The six successive aqueous extracts thus obtained were examined polarimetrically, and the amount of material dissolved in them was also quantitatively determined. The results were as follows :—

| Fraction of Extract. | $\alpha_D$ in a 2 Dcm. Tube. | Amonnt of Substance contained in 10 C.c. of Solution. | $[\alpha]_D$. |
|---|---|---|---|
| I. ............ | $-23^\circ\ 9'$ | 1·4710 gramme. | $-78{\cdot}7^\circ$ |
| II. .......... | $-10^\circ\ 30'$ | 0·5988 ,, | $-87{\cdot}7^\circ$ |
| III. .......... | $-7^\circ\ 40'$ | 0·3541 ,, | $-108{\cdot}3^\circ$ |
| IV. .......... | $-5^\circ\ 57'$ | 0·2520 ,, | $-118{\cdot}1^\circ$ |
| V. ............ | $-5^\circ\ 0'$ | 0·1990 ,, | $-125{\cdot}6^\circ$ |
| VI. ........ | $-3^\circ\ 22'$ | 0·1362 ,, | $-123.6^\circ$ |

From the results thus afforded by the fractional solution of the above-described product it is apparent that the latter is not at all homogeneous, but that it represents a complex mixture.

Some further information regarding the character of the glucosidic product was obtained by the following method of examination. About 15 grammes of the product were mixed with water, and the mixture extracted many times with amyl alcohol. The greater part of the product was thus taken up by the amyl alcohol, while a small portion remained dissolved in the aqueous liquid. The amyl alcohol solution was then successively and exhaustively extracted with aqueous solutions of ammonium carbonate, sodium carbonate, and potassium hydroxide, each of these extracts being subsequently acidified, and the liberated product quickly taken up by amyl alcohol. The original material thus yielded five products differing essentially from one another in their properties, but all of them uncrystallisable, namely:—

*Product 1.*—This consisted of the material which could not be removed from its aqueous solution by means of amyl alcohol. It was small in amount, and, as it might have contained traces of sugar, was not submitted to hydrolysis.

*Product 2.*—This material was extracted from the amyl alcohol solution by means of aqueous ammonium carbonate. It was small in amount, but on hydrolysis with dilute sulphuric acid yielded a little sugar.

*Product 3.*—This material was extracted from the amyl alcohol solution by means of aqueous sodium carbonate. It represented about 40 per cent. of the total material, and, when hydrolysed, yielded an abundance of a reducing sugar from which *d*-phenylglucosazone was prepared.

*Product 4.*—This material was extracted from the amyl-alcohol solution by means of aqueous potassium hydroxide. It was rather small in amount, and was not glucosidic.

*Product 5.*—This consisted of the non-acidic and non-phenolic material which remained dissolved in the original amyl alcohol solution. It amounted to about 30 per cent. of the total material, and did not appear to be altered by prolonged boiling with dilute sulphuric acid, no sugar having been obtained from it.

With consideration of the results of the above described experiments it is obvious that the material obtained by Bourquelot and Vintilesco (*loc. cit.*), and described by them as "a new principle of glucosidic nature," under the title of "oleuropein," is not a homogeneous or individual substance, but a very complex mixture of amorphous products. The components of this amorphous mixture, as obtained from olive leaves, differ widely from each other in solubility, in optical rotatory power, and in their behaviour towards alkalis, and, furthermore, some of them are not even glucosidic.

Notwithstanding the detailed method of preparation, the above-mentioned authors have, in fact, given no evidence of the homogeneity of the material obtained by them, and their conclusion that it was relatively pure appears to have been based merely on the observation that the products obtained from olive leaves and olive fruit respectively possessed pretty nearly the same properties.

In conclusion, it may, therefore, be definitely stated that, although different parts of the olive tree yield products from which glucose may be obtained on hydrolysis, as had previously been ascertained by us, these products are amorphous and of more or less complex composition. No product, however, of a glucosidic nature appears as yet to have been isolated from any part of the olive tree which is entitled to such a distinctive name as would indicate it to be a homogeneous or individual substance.

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